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The Effect of Sampling Time and Flow Rates on the Bioefficiency of Three Fungal Spore Sampling Methods

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ABSTRACT. The influences of sampling time and flow rates on total recovery rate of airborne fungal spores were evaluated in the laboratory test chamber by three sampling methods: AGI-30 all-glass impingers, the Nuclepore filtration and elution method, and the gelatin filters. In the test system, a Pitt-3 generator was used to generate the spores of *Penicillium citrinum*. The real-time number concentration of fungal spores was measured by a TSI aerodynamic particle sizer to determine the total recovery. The results demonstrated that total recovery of spores collected by the AGI-30 impinger was in the range of 4% to 24%. Moreover, the average total recoveries of spores collected by Nuclepore and gelatin filters were found to be in the range of 50% to 75%, and 50% to 90%, respectively. The observed low total recovery for the AGI-30 impinger may be due primarily to the biological stress during sampling process. The relative survival of fungal spores collected in AGI-30 impingers became lower as the sampling time and flow rates increased. However, no significant influences of sampling time and flow rates on the total recovery of both the Nuclepore filtration and gelatin-filter methods were observed. In conclusion, it was found that filtration methods could perform much better than impingers for sampling airborne fungal spores. AEROSOL SCIENCE AND TECHNOLOGY 28: 511–522 (1998) **© 1998 American Association for Aerosol Research**

INTRODUCTION

Recently, health evaluation of bioaerosols has become an important issue. Airborne microorganisms in indoor and outdoor environments, from either natural or industrial sources, may produce ill effects on humans, ranging from mild irritation to diseases. Therefore, it is important to evaluate the composition and concentration of airborne microorganisms in contaminated environments to ensure the health of workers and the public. Samplers for microbiological aerosols have been designed similar to general aerosol samplers and also have to be evaluated in a similar way, but an additional vital factor to be determined is the capacity of the samplers to preserve the culturability of the airborne microorganisms (Henningson and Ahlberg, 1994).

Both microorganisms and biologically inert particles have been used as test aerosols among which the most common types of microorganisms have been bacteria (May and Harper, 1957; Adams et al., 1992;

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Juozaitis et al., 1994; Qian et al., 1995; Lighthart and Shaffer, 1997). However, fungal spores were much less frequently evaluated (Reponen et al., 1997). Fungal spores vary greatly in size, but most are in the range of 2–50 μm, which are larger than actinomycetes and other bacterial spores and generally smaller than pollens. For the great majority of dry-spored fungi, air is their natural dispersal medium, and accordingly they have evolved various mechanisms that enhance their effective dispersal and survival in air (Madelin and Madelin, 1995). The spores dispersed by air movement are usually hydrophobic (Levetin, 1995), therefore the collection efficiency by liquid medium may be lower than that of hydrophilic bacterial aerosols. Since the physical and biological properties of fungal spores are quite different from those of bacterial aerosols, the evaluation is needed to assess the sampler performance for collecting fungal spores.

For the sampler assessment of fungal aerosols, most studies were conducted in natural environments (Blomquist et al., 1984; Kang and Frank, 1989; Morey, 1990; Verhoeff et al., 1990; Thorne et al., 1992; Cage et al., 1996; Mehta et al., 1996) and very few evaluations were performed in laboratory chambers (Silas et al., 1986; Buttner and Stetzenbach, 1993). However, evaluations in a natural environment provide very limited information with uncontrolled or unknown natural aerosol concentrations, particle sizes, and flora (Henningson and Ahlberg, 1994). It was believed that field measurements are not easily compared to each other because of many uncontrolled or nonconsistent environmental factors (Nevalainen et al., 1993). However, laboratory production of test fungal spores would be preferable to aim toward controllable reproduction of required size, biological content, and level of concentration (Griffiths and DeCosemo, 1994). Therefore, a test system in our laboratory was established to assess the performance of the bioaerosol samplers.

The overall sampling efficiency of samplers with different design may differ significantly from each other due to the different physical collection efficiency and the stress imparted to the microorganisms. There are many different factors influencing the microbial collection and survival in bioaerosol samplers. Aerosol concentration and composition, inlet orientation, aerosol charge, particle desiccation and shear forces, wind speed, particle breakup, sampling time, and sampling flow rate may affect the number of collected microorganisms as well as their culturability and culturability (Macher and Willeke, 1992; Nevalainen et al., 1993; Grinshpun et al., 1994; Cox and Wathes, 1995). Among these factors the most important issues are considered to be the selection of sampler, sampling time, and sampling flow rate (Nevalainen et al., 1993).

In this study, a test system in a laboratory was established for the generation of fungal spores. The performances of three sampling methods including AGI-30 impingers, Nuclepore filtration and elution method, and the gelatin filters were compared, and the influences of sampling time and flow rate for viable fungal aerosols also were evaluated.

MATERIALS AND METHODS

Test System

The schematic representation of sampling method performance for viable fungal spores is shown in Fig. 1. A Pitt-3 generator was used to generate airborne fungal spores into a test chamber of 30 cm in diameter. The acoustic-vibration aerosol generator, Pitt-3 model, was initially designed, fabricated, and characterized for the resuspension of inhalable particles from bulk cotton dust (Weyel et al., 1984; Frazer et al., 1986, 1987) and compost dust (Frazer et al., 1993). The generator was constructed around a loudspeaker whose energy is transferred into an air column through latex rubber dams. This action tumbles the bulk dust, and small particles are loosened that can then be carried out of the column with the air passing through it. The Pitt-3 generator has been used to generate dry fungal spores (Sorenson et al., 1987; Buttner and Stetzenbach, 1993). In our test system, the generator has

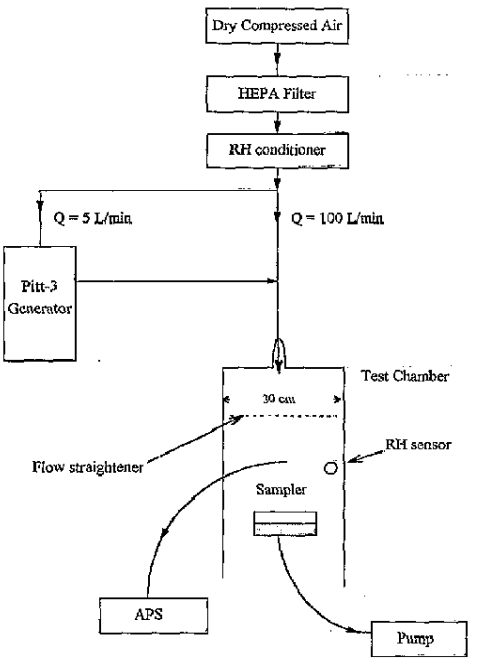

FIGURE 1. Schematic drawing of the experimental system and measurement system. Spores of *P. citrinum* were introduced into the test chamber from the Pitt-3 generator. An APS was used to measure real-time number concentrations and size distribution of fungal spores throughout the run. The test system was located in a chemical hood through which the exhausted air was vented outside.

a 12-cm diameter and 36-cm length of Plexiglas tube, with vibration provided by rubber dam material over an 18-cm speaker, powered at 60 Hz and 2 to 6 volts. Generally, for each trial, a dry aerosol of spores was generated inside the sealed Plexiglas column by acoustic vibration. Dry and filtered compressed air conveys spores from the Pitt-3 generator to the test chamber at a flow rate of 5 L/min. A purified filtered compressed air at a flow rate of 100 L/min was introduced into the test chamber to dilute the aerosol concentration and offer sufficient air stream for sampling.

A TSI aerodynamic particle sizer (APS, Model 3310A, TSI, Inc., St. Paul, MN) was used to determine real-time number concentration and size distribution of fungal spores throughout the run in order to determine total recovery (TR) of spores collected by the test sampler. The APS is capable of measuring particles in the size range of 0.5 to 30 μm and operating at an airflow rate of 5 L/min. To determine the relative survival (RS), the test sampler was placed under the flow straightener in the test chamber together with the reference sampler, with the AGI-30 impinger set at a flow rate of 12.5 L/min, which was widely applied in field measurement. During the tests the concentrations measured by APS ranged from 10^4 to 10^8 particles/m^3, which represented the medially to highly contaminated environments (Malmberg et al., 1988; Rahkonen et al., 1990; Kotimaa et al., 1991; Rautiala et al., 1996; van der Werf, 1996; Nielsen et al., 1997). By controlling the amounts of harvested spores and the volume of the speaker, the concentrations could be maintained around 10^6 particles/m^3 for at last three hours. The test system was located in a chemical hood through which the exhausted gas was vented outside.

Bioefficiency Calculation

The bioefficiency of the samplers was estimated by determining the relative survival, RS (Henningson and Ahlberg, 1994) and the total recovery, TR (Juozaitis et al., 1994). When the CFU concentration measured by the test sampler, C_{test}, was compared with the CFU concentration measured by an AGI-30 impinger operated simultaneously at 12.5 L/min, C_{ref}, the relative survival, RS, was given by

$$RS = C_{test}/C_{ref}.$$

When the CFU concentration measured by the test sampler, C_{test}, was compared with the total number concentration of spores measured by APS, C_0, the total recovery of the spores, TR, was given by

$$TR = C_{test}/C_0.$$

Test microorganism

Spores of *Penicillium citrinum* Thom (CRCC 33168) were used in the study. This organism is a common fungal isolate in Taiwan, and the spores (conidia) are subspheroidal to spheroidal, 2.0–3.6 μm in physical diameter, and finely roughened (Tzean et al., 1994). Strains of *P. citrinum* were cultured on malt extract agar (MEA, pH 4.7, Difco Laboratories, Detroit, MI) and were incubated at room temperature (ca. 25°C) for seven days before harvest. The harvested spores were scraped by a loop and placed onto the rubber dam inside the Pitt-3 column and then prepared for generation. The culturability of harvested spores was determined by the method described in the previous study (Griffiths et al., 1996).

Test Samplers

The AGI-30 (Ace Glass Inc.) is an all-glass impinger with a 30-mm jet-to-plate distance. It was developed from the Porton impinger for sampling microorganisms (May and Harper, 1957) and proposed as a reference sampler (Brachman et al., 1964). Suction is applied to the small side arm and draws air in through inlet tube curved to simulate particle collection in the nasal passage and down through the impinging jet. The jet nozzle has been raised above the liquid surface in an attempt to get a more gentle impaction surface than the glass bottom of the flask. Generally, 20 ml of sterile deionized water with 0.01% Tween 80 and 0.005% antifoam A (Sigma Chemical Co., St. Louis, MO) was injected into each autoclaved AGI-30 impinger (Thorne et al., 1992). The antifoam was added to reduce foaming and to prevent excessive fluid loss (Buchanan et al., 1972; Lembke et al., 1981).

A Nuclepore filter consists of a polycarbonate membrane with straight-through pores of a uniform size (0.01 to 14-μm pore size) and is widely used for particle analysis using surface analytical techniques such as light and electron microscopy (Hobbie et al., 1977; Palmgren et al., 1986a, 1986b). In this study, filters with a 0.4-μm pore size and a 37-mm diameter supported by cellulose pads were loaded into open-face and three-piece plastic cassettes. Filters and support pads were autoclaved, and plastic cassettes were sterilized with ethylene oxide before sampling.

The gelatin filter (Sartorius, Gottingen, Germany) has been specially designed for detection and analysis of airborne microbes. Each sterile gelatin filter (3.0-μm pore size, 80-mm diameter) was placed in a sterile filter holder by carefully letting the filter slide out of the pocket onto the filter support of the aluminum filter holder. The filter could dissolve on the agar surface because of the moisture in the agar culture medium or in sterile liquid at the temperature of 35–40°C.

Sampling Time and Flow Rate

To evaluate the influence of sampling time on the collection of viable airborne fungal spores, the selected sampling time included 1, 2.5, 5, 15, 30, 45, and 60 min. For the AGI-30 impinger, the sampling flow rates consist of 4.7, 7.1, 9.4, 12.5, and 14 L/min, and the corresponding velocity through the nozzle was 100, 150, 200, 265, and 300 m/s, respectively. The samples were collected by calibrated Andersen vacuum pumps. The sampling flow rates of Nuclepore filters were selected to be 1, 1.5, 2, and 4 L/min, and the corresponding sampling velocity was 2, 3, 4, and 8 cm/s, respectively. The samples were collected by calibrated SKC personal sampling pumps. The airflow rate for the gelatin filters is recommended to be approximately 2.7 ± 0.5 L/min/cm^2 by the manufacturer. In our tests the airflow rate was reduced to the range of 0.12 to 0.72 L/min/cm^2, and the selected sampling flow rates were 5, 15, and 30 L/min, which were widely used by industrial hygienists and matched with the sampling velocity selection for Nuclepore filtration. The corresponding sampling velocity was 2, 6, and 12 cm/s, respectively. The 5 L/min samples were collected by calibrated SKC personal sampling pumps, while the 15- and 30 L/min samples were collected by calibrated Andersen vacuum pumps. At least

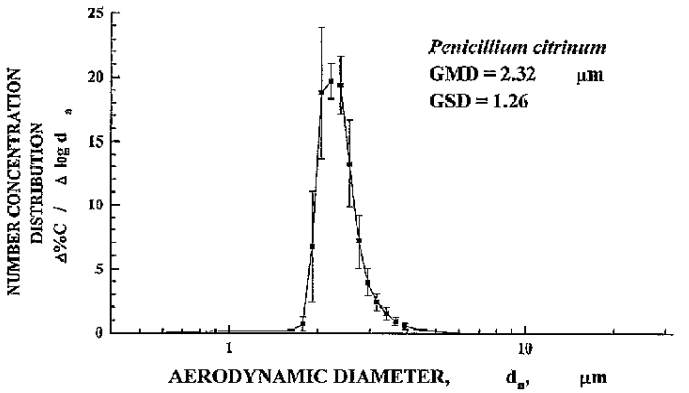

FIGURE 2. The particle size distributions of *P. citrinum* spores in the test chamber measured by APS. Each error bar represents one standard deviation on the mean of 30 samples. More than 95% of the aerodynamic diameters of spores were in the range of 1.8 μm to 3.6 μm.

triplicate tests were performed for each set with different sampling time and flow rates.

Sample Processing

After sampling, the spores collected by the filtration methods needed to be eluted. The Nuclepore filter was removed into a test tube containing 10 ml of sterile deionized water, and the spores were detached from the filter by vertexing the tube for 30 s (Qian et al., 1995). The gelatin filter was dissolved in 20-ml sterile deionized water in a 90 × 15-mm sterile petri dish in a 37°C incubator for 15 min. Suspensions of the samples collected by AGI-30 impingers and filters then were vertexed, and triplicate 0.2-ml samples of serial 10-fold dilution were plated onto MEA. All samples were processed within 30 min to avoid the decay of the culturability. Agar plates were incubated for two to three days at room temperature (ca. 25°C) before counting.

CFU Counting

Colony forming unit (CFU) counting was done on plates containing between 30 and 300 colonies (Lembke et al., 1981; Thorne et al., 1992). The lower limit (30 colonies) is necessary to obtain sufficient statistical power for comparison purposes. The upper limit (300 colonies) is the maximum range in which one could easily count and differentiate colonies (Jensen et al., 1994; Chang et al., 1994). Airborne spore concentrations are determined by multiplying the CFU by the dilution factor and by the elute volume and dividing by the volume of serial dilution material plated (0.2 ml) and the volume of sampled air that is calculated from the sampling time and flow rate.

RESULTS AND DISCUSSIONS

Characteristics of Aerosolized Penicillium Spores

The aerodynamic particle diameter of the target aerosol is one of the most important physical factors that determine the stage collection efficiencies of inertial and filtration devices. By using APS, the test aerosol, spores of *P. citrinum* generated from the Pitts-3 generator, was demonstrated with the aerodynamic particle diameter to be in the range of 1.8 to 3.6 μm (Fig. 2). It was found that the average values of GMD and GSD of *P. citrinum* spores were 2.32 μm and 1.26, respectively. The particle size of *P. citrinum* spores used in this evaluation was similar to those of *P. chrysogenum* (Buttner and Stet-

zenbach, 1993), *P. brevicompactum* (Reponen et al., 1996), ambient mixed *Penicillium* spp. (Lin and Li, 1996), and indoor mixed *Penicillium* spp. (Reponen, 1995).

Besides the characteristics of the size distributions, the culturability of the generated spores is another important issue we should consider. Generally considering, the culturability of aerosolized microorganism may be affected by the culturability of the source of microorganism, the aerosolization procedure, and the duration of the aerosolized microorganism. In our current evaluation, all of the harvested spores were found to be culturable (data not shown). In addition, the pattern of near-natural aerosolization and the strong resistance of airborne spores also confirm the culturability of the airborne spores in the test chamber (Levine, 1979). Therefore, it was believed that the total recovery of the spores in this investigation depends primarily on the physical efficiency and biological stress of the collection methods, not the culturability of the aerosolized spores.

Bioefficiency of Test Samplers

AGI-30 impinger: Bioefficiency analysis at different sampling time demonstrated that TR of the AGI-30 impinger at the flow rate of 12.5 L/min, was 24 ± 9% for 1-min samples and decreased to 3.9 ± 2.5% for 60-min samples (Fig. 3b). The comparisons of AGI-30 impingers at different flow rates indicated that RS decreased as sampling time increased at the low flow rates, 4.7 L/min (Fig. 3a). RS of AGI-30 impinger at 4.7 L/min was found to be as high as 400% for the 15-min samples. On the other hand, RS of AGI-30 impinger at 9.4 L/min was found to be slightly higher than 100%, no matter how long the sample was collected. In a similar way, RS of the AGI-30 impinger at 14 L/min was found to be slightly lower than 100%. The means of RS were 260%, 180%, 140%, and 95% at the flow rate of 4.7, 7.1, 9.4, and 14 L/min, respectively (Fig. 3c). RS was found to decrease as sampling flow rate increased.

It was believed that both sampling time and flow rates could affect the collection efficiency and the culturability of collected bioaerosols for the AGI-30 impinger (Juozaitis et al., 1994; Terzieva et al., 1996; Lin et al., 1997). Concerning sampling time, the collected percentage of 1.60-μm PSL particles still remained over 90% for 60-min sampling (Lin et al., 1997). In regard to sampling flow rate effects, the change of impinger flow rates would change the cutoff diameter (Asking and Olsson, 1997) and bubble pattern (Grinshpun et al., 1997). For the AGI-30 impingers, the calculated cutoff

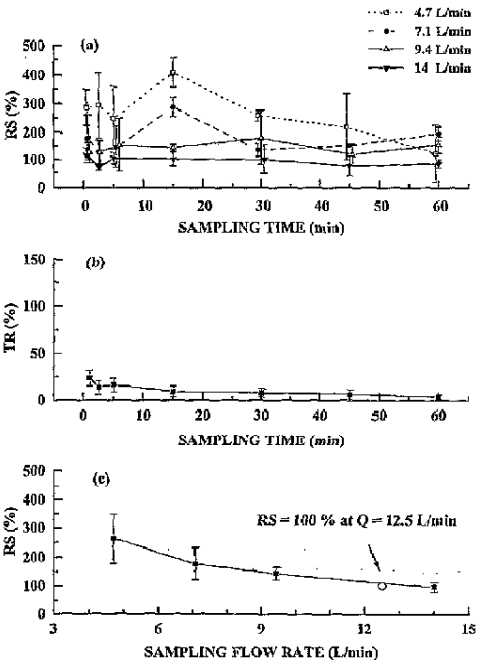

FIGURE 3. The performance of AGI-30 at different sampling time and flow rates: (a) relative survival, RS (CFU concentration divided by CFU concentration measured simultaneously by an AGI-30 at flow rate of 12.5 L/min) of samples collected at four sampling flow rates. Each error bar represents one standard deviation on the mean of at least three tests; (b) total recovery, TR (CFU concentration divided by concentration measured by APS) of samples collected at 12.5 L/min. Each error bar represents one standard deviation on the mean of at least 30 tests; (c) average RS over different sampling time at four sampling flow rates.

diameter will decrease as flow rate increases (Nevalainen et al., 1992; 1993). In our current study the aerodynamic particle diameter of fungal spores was large enough that there was sufficient inertia to impinge fungal spores into collection solution when the flow rate was as low as 4.7 L/min. Therefore, sampling time and flow rates would not significantly influence the collection efficiency for fungus collection.

Another important issue with which we are concerned was the hydrophobicity effect of fungal spore. The physical collection efficiency of hydrophobic spores may be reduced, because spores may bounce into the effluent flow or reaerosolize when a spores-entrained bubble bursts. In our current investigation, a wetting agent, Tween 80, was added into the collection solution for enhancing the suspension of the spores during impingement. However, the influence of spore hydrophobicity and reaerosolization on impinger collection efficiency still needs further evaluation.

At the low flow rates, such as 4.7 L/min, the less intense bubbling in the liquid was observed to reduce solution evaporation and spore reaerosolization. Moreover, the evaporation rate of collection solution in our study was reduced by adding antifoam agent so that reaerosolized spores from the collection suspension should be minimized. The physical collection efficiency of spores should be higher than that of PSL and bacterial particles (Grinshpun et al., 1997), and TR values should be associated primarily with the biological stress that would affect the survival of collected spores.

At the flow rate of 12.5 L/min, TR was observed to be 24% for 1-min samples. The low level of TR value was far below 100% (collection efficiency), which should be primarily caused by the biological stress. For AGI-30 impingers, the biological stress during sampling consists of impinging action through the nozzle of the inlet tube, impaction onto the bottom of the collection bottle, and contact effect with collection solution (Juozaitis et al., 1994; Terzieva et al., 1996). The biological stress during impingement was found to be so destructive that about three-fourth of the spores lost their culturability after being collected.

As sampling time prolonged, TR decreased from 24% to 4% after 60-min impingement, which indicated sampling time prolongation might increase the biological stress onto the collected fungal spores. Regarding sampling flow rate effects, the increase of the impinger flow rate should increase biological stress of the bioaerosols (Juozaitis et al., 1994). In our study, TR of fungal spores demonstrated biological stress became higher as sampling flow rate increased, because the high velocity through the nozzle should enhance impaction stress onto fungal spores. As sampling time prolonged, the contact effect and stress to damage collected spores might become comparable so that RS decreased as sampling time increased at the flow rates of 4.7 and 7.1 L/min (Fig. 3a).

Nuclepore filter: Our study was the first investigation to evaluate the effect of sampling time and flow rates on the bioefficiency of Nuclepore filters for collecting fungal spores. The average values of TR (%) were 61 ± 28, 53 ± 31, 72 ± 31, 64 ± 20, 63 ± 17, 68 ± 14, and 59 ± 26 at the sampling time of 1, 2.5, 5, 15, 30, 45, and 60 min, respectively (Fig. 4b). Moreover, the average values of TR (%) were 68 ± 28, 56 ± 26, 65 ± 29, and 68 ± 24 at the flow rate of 1, 1.5, 2, and 4 L/min, respectively (Fig. 4c). It was demonstrated clearly that both sampling time and flow rates did not significantly influence the performance of Nuclepore filters for collecting fungal spores.

For Nuclepore filters, the particle diameter of fungal spores was much larger than the 0.4-μm pore size, and the spore penetration through the filter should be negligible. Therefore, TR values should be related primarily to the biological stress during sampling.

The greatest biological stress during filtration may be impaction and dehydration effects caused by the large volume of air that may pass over the collected bioaerosols (Crook, 1995). In our study, sampling time prolongation was found not to reduce TR of fungal spores. Therefore, it was indicated

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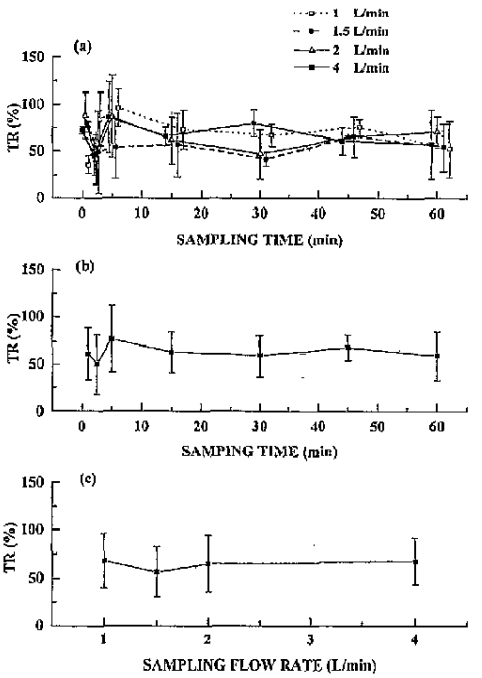

FIGURE 4. The performance of the Nuclepore filtration and elution (NFE) method at different sampling time and flow rates: (a) total recovery, TR of samples collected at four sampling flow rates. Each error bar represents one standard deviation on the mean of at least three tests; (b) average TR over different sampling flow rates at different sampling time; (c) average TR over different sampling time at four sampling flow rates.

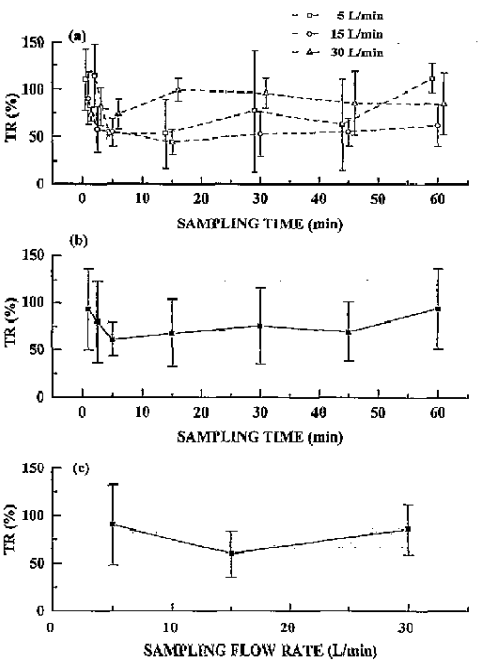

FIGURE 5. The performance of gelatin filters at different sampling time and flow rates: (a) total recovery, TR of samples collected at three sampling flow rates. Each error bar represents one standard deviation on the mean of at least three tests; (b) average TR over different sampling flow rates at different sampling time; (c) average TR over different sampling time at three sampling flow rates.

that dehydration during sampling did not significantly affect culturability of fungal spores that are resistant to desiccation. The primarily biological stress would occur as soon as the spores impacted onto the surface of the Nuclepore filter. In comparison with AGI-30 impingers, the Nuclepore filters had much higher TR values that agreed well with those found in the swine confinement for fungal particles (Thorne et al., 1992).

Gelatin filter: The average TR (%) values of gelatin filter were observed to be 93 ± 43, 80 ± 40, 61 ± 18, 68 ± 36, 76 ± 41, 70 ± 32, and 94 ± 43 at the sampling time of 1, 2.5, 5, 15, 30, 45, and 60 min, respectively (Fig. 5b). Moreover, the average TR (%) values was observed to be 90 ± 42, 60 ± 24, and 86 ± 26 at the flow rate of 5, 15, and 30 L/min, respectively (Fig. 5c). Both sampling time and flow rates did not have significant influences on the gelatin filter performance for collecting fungal spores.

The collection efficiency has been observed to be 99.9% for particles of 0.5 to 3 μm, despite a filter pore size as large as 3 μm (Rotter and Koller, 1973) and comparable to that of a cellulose acetate membrane filter (Macher and First, 1984). Therefore, the influence of physical factors on sampling performance then could be negligible for gelatin filters.

For fungal spores, filtration time was

found not to influence the TR values in our study. For natural bacterial aerosols, longer filtration time was observed to result in relatively lower recovery as the bacterial yield of 60-min filtration became 70% of that of 5-min filtration (Rotter et al., 1973). In general, gelatin filters are considered to be unsatisfactory for collecting airborne bacteria because during extended sampling the gelatin dried out, which placed additional dehydration stresses on the collected microorganisms (Crook, 1995). In our study, fungal spores were found to be more resistant against dehydration stress than airborne bacteria during gelatin filtration.

The physical collection efficiency of filtration was found to be comparable to that of impingers for collecting fungal spores. Therefore, the observed higher TR values of filtration than impingement should be associated with the lower biological stress by filtration, which was related to much lower sampling velocity. In addition, contact stress caused by the solid filter could be lower than liquid collection medium.

CONCLUSIONS

The observed low level of total recovery of fungal spores collected in AGI-30 impingers may be due primarily to the higher biological stress during sampling process. The total recovery of fungal spores collected in AGI-30 impingers became lower as the sampling time and flow rate increased. Furthermore, the sampling time and flow rate did not have significant influences on the recovery of fungal spores collected on the Nuclepore or gelatin filters. For sampling airborne fungal spores, filtration methods could perform better than impingers and less sensitive to variant sampling time and flow rates. Further sampling efficiency evaluations of fungi with other spore form are needed.

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